Characterizing Disease Related Mutations in Proteins Involved in Liquid-Liquid Phase Separation

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LIQUID-LIQUID PHASE SEPARATION (LLPS)

LLPS is a physicochemical process where a liquid forms two phases: a condensed phase and a diluted phase.

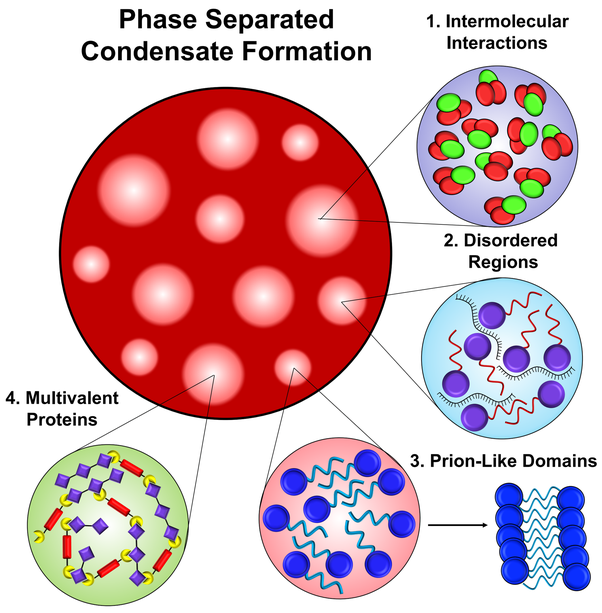


Fig 1. Through several forms of molecular interactions (1-4), LLPS leads to a diluted (dark red) and condensed phase (light red) also known as a condensate.

Protein Properties that cause LLPS

1. Hydrophobic & Electrostatic Interactions
2. Intrinsically Disordered Regions (IDRs)
3. Prion-Like Domains (PLDs)
4. Multiple Domains
5. Higher Expression

LLPS in the Cell

- Nucleoli
- Stress Granules
- Signaling Clusters
- Centrosomes

Mutations in LLPS Proteins

Mutations that alter physicochemical properties of a protein can impact its probability of forming a phase-separated condensate.

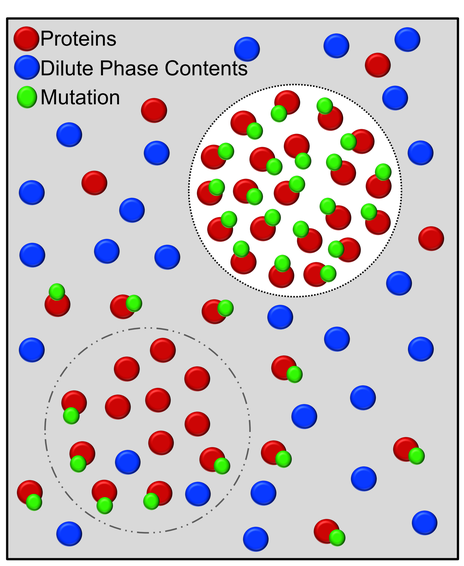
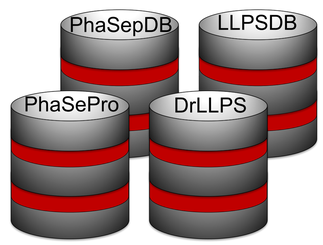


Fig 2. Mutations can cause LLPS proteins to form condensates (Gain-of-Function/GOF, top half) or disrupt already formed protein condensates (Loss-of-Function/LOF, bottom half).

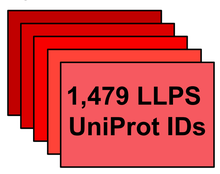
MUTATION DATABASE GENERATION

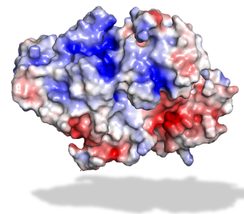
1. LLPS Protein Consensus

LLPS Protein Databases (DB)

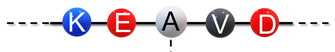


Experimentally Validated LLPS proteins in 2+ DBs





Wild Type Protein (FASTA) sequences



2. Disease Mutation Database Selection + Filtering

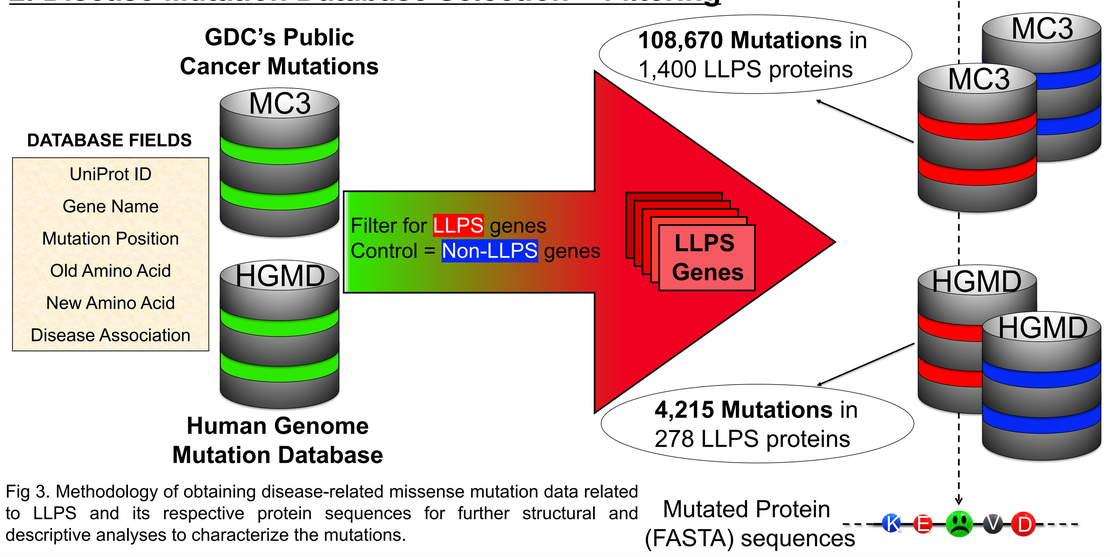


Fig 3. Methodology of obtaining disease-related missense mutation data related to LLPS and its respective protein sequences for further structural and descriptive analyses to characterize the mutations.

SYSTEMATIC STRUCTURAL ANALYSES

1. Hydrophobicity Changes

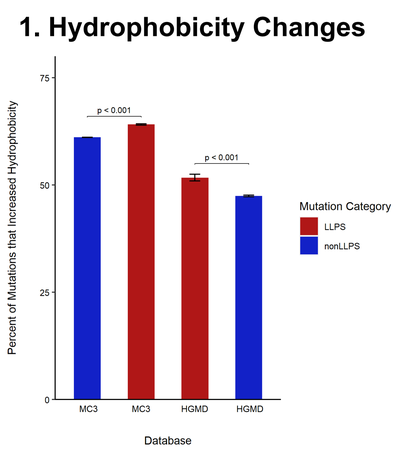


Fig 5. Percent of mutations in each database that increased hydrophobicity of the protein as measured with amino acid hydrophobicity rankings provided by Sahni *et al.* (2015)

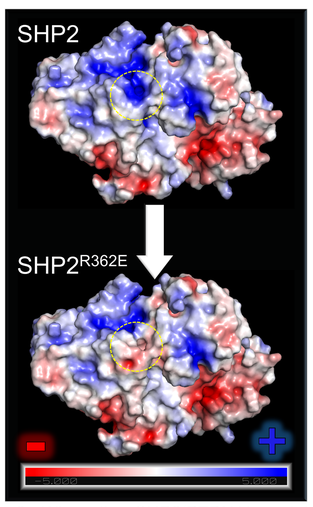


Fig 6. When the SHP2/PTPN11 protein is mutated (R362E), there is a change of charge in the area of mutation. PDB: 4DGP

2. Disordered Regions

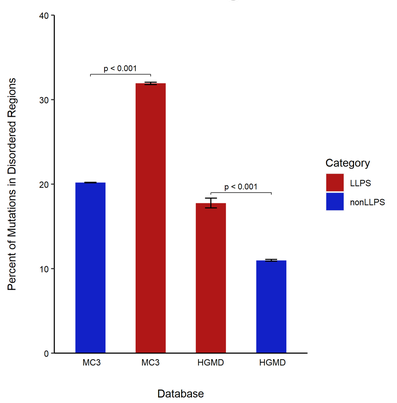
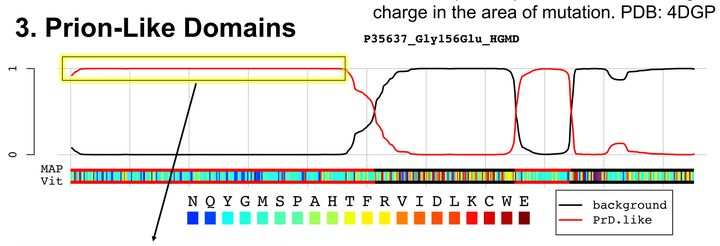


Fig 7. Mutations occurring in disordered regions were predicted using IUPred2A for both databases.

3. Prion-Like Domains



Mutations significantly occur in a PLD for LLPS genes in HGMD. (p = 5.4978e-18)

Fig 8. The PLDs of each mutated protein sequence in HGMD were predicted using PLAAC.

Significantly Enriched LLPS Genes

PTEN	MAP2K1
CDKN2A	SH2B1
CREBBP	LMNA
ATRX	ATM
FUS	…
SOD1	SOS1
LRRK2	ACTB
KIT	PRPF31
PTPN11	APOA1
MAPT	

4. Change in Expression

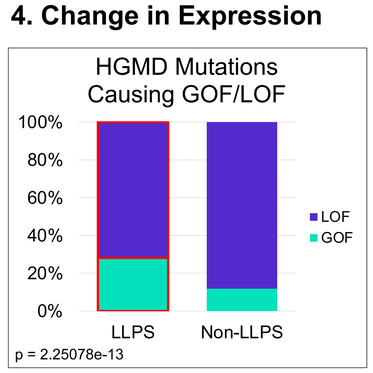


Fig 9. If data was available by Bayrak *et al.* (2021), mutations were labeled as GOF or LOF with regards to gene expression.

LLPS Related Diseases

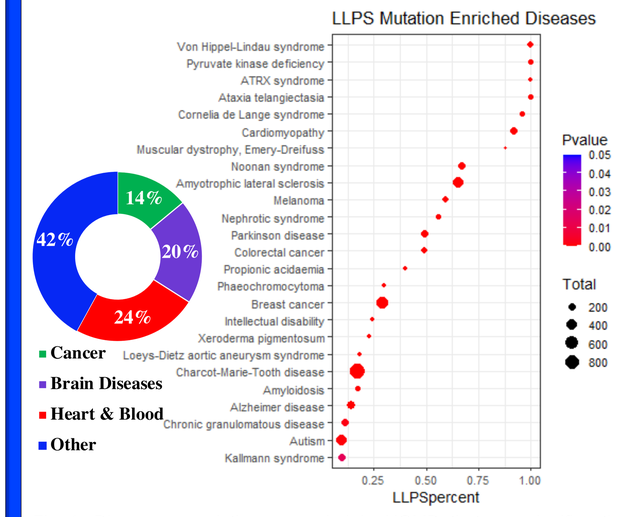


Fig 4. Disease associations recorded in HGMD that are significantly enriched with mutations in LLPS genes.

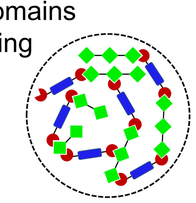
CONCLUSIONS

We were able to compile a database of disease mutations occurring in LLPS genes and characterize them through several systematic analyses. So far, we have observed that mutations in LLPS genes are more likely to increase the hydrophobicity of its proteins and to occur in IDRs and PLDs.

Overall, we have promising data and findings for constructing a detailed profile for our disease mutations in LLPS genes. This profiling will help us characterize and understand the roles of mutations that affect LLPS in diseases like cancer and neurodegeneration.

NEXT STEPS

- ❑ Complete remaining structural analyses for HGMD and MC3
  - Polarity/Charge Changes
  - Solvent Accessibility
  - Secondary Structures
  - Mutations in Protein Domains
  - Stability & Protein Folding



- ❑ Protein-Protein Interactions
  - PPI Networks
    - Clients: Proteins involved in LLPS
    - Scaffolds: Proteins that drive LLPS

Acknowledgements

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References

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